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THE INFLUENCE OF STRYCHNIN, CAFFEIN, CHLORAL, ANTIPYRIN, CHOLESTEROL, AND LACTIC ACID ON PHAGOCYTOSIS.*

AARON ARKIN.

(From the Memorial Institute for Infectious Diseases, Chicago.)

There are three essential factors concerned in bacterial phagocytosis on which chemical substances may act so as to increase or depress phagocytic activity, namely: (1) the leukocytes, (2) the opsonins, and (3) the bacteria.

The effects of a variety of substances on phagocytosis have been studied and a brief statement of the results may be of interest.

THE ACTION OF SALTS ON PHAGOCYTOSIS.

That a large number of salts inhibits phagocytosis was demonstrated by Hektoen and Ruediger, who found that $CaCl_2$, $BaCl_2$, $SrCl_2$, $MgCl_2$, K_2SO_4, $NaHCO_3$, $Na_3C_6H_5O_7$, $Na_2C_2O_4$, $K_4Fe(CN)_6$ all neutralize or bind the opsonins so that these cannot act on the bacteria. Eggers studied the effects of salts normally present in the serum and found that $BaCl_2$ and NaF have a marked toxic action on the leukocytes. $MgCl_2$ has a stimulating action on the leukocytes.

Calcium has a distinct stimulating action on phagocytosis of carbon particles as shown by Hamburger. A 0.005 per cent solution causes a rise of phagocytosis of 22 per cent (calcium chlorid). Furthermore, calcium has a marked positive chemotactic action, increasing the movements of the phagocytes not only *in vitro* but also in the living body. The action appears to be a specific property of this element and is not dependent on its electrical charge.

THE EFFECT OF REACTION.

One would expect opsonins to exert their maximum activity in normal serum, i.e., in a slightly alkalin medium. Hamburger and Hekma found that a very slight acid reaction is sufficient to inhibit phagocytosis to a marked degree. When the reduction of the OH ions reached 5 per cent or when they were increased 15 per cent there was a marked effect. Noguchi, however, claims that normal opsonins act best in neutral reaction and that no opsonization occurs in a serum containing more alkali than 1.6 c.c. of n/20, or more acid than 0.5 c.c. of n/20 per 1 c.c. of serum. Eggers has found that the maximum action occurs at normal (alkalin) reaction of the serum, agreeing with Hamburger and Hekma.

THE EFFECT OF ANTISEPTICS.

The action of some surgical antiseptics on phagocytosis was studied by Manwaring and Ruh. Carbolic acid causes from the first a decrease of phagocytosis, with cessation at 0.5 per cent. Mercuric chlorid in weak solution (less than 1/120 per cent)

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causes transient stimulation followed by depression. Neuber found that mercuric chlorid injected into animals has the same effect. Hektoen and Ruediger showed that formalin causes marked inhibition of phagocytosis in solution of 1 to 5,000.

I have found that sodium iodoxybenzoate has a marked stimulating action on the phagocytosis of streptococcus and staphylococcus by human leukocytes in the presence of human serum. This substance has a distinct germicidal action which is dependent on the oxygen attached to iodin in the molecule. Likewise, the stimulating effect on phagocytosis is due to the oxygen, since sodium iodbenzoate, which contains no oxygen, has no effect. The iodoxybenzoate probably acts on the opsonin to produce a more active combination. That the oxygen is the important factor is also indicated by the fact that potassium cyanide, which depresses oxidation, has a marked inhibitory influence on phagocytosis *in vitro*. These experiments suggest a relationship between oxidation and the process of phagocytosis.

THE ACTION OF RADIUM ON PHAGOCYTOSIS.

Chambers and Russ found that the alpha and beta rays from comparatively small quantities of radium have a direct bactericidal action. The polymorphonuclear leukocytes of human blood suffer a reduction in their phagocytic power and are eventually destroyed when exposed to the alpha rays. The opsonin in normal serum is destroyed by these rays. Reiter found that the emanations *in vitro* stimulate the phagocytic activity of the blood cells in some experiments to 30 per cent. Under certain circumstances the emanations may so act on the bacteria as to protect them from phagocytosis.

Bouchard, P. Curie, and Balthazard found that a leukopenia occurs in animals poisoned with radium emanations. Klecki recently found that radium emanations have a different effect on the phagocytosis of different bacteria. Radium increases the phagocytosis of the colon bacillus and the staphylococcus, and reduces the phagocytosis of the tubercle bacillus. The increase of phagocytosis is chiefly through the action on the phagocytes, and to a lesser degree on the bacteria.

ACTION OF COLLOIDAL METALS.

Bossan and Marcelet found that colloidal suspensions of gold, copper, silver, and platinum have a marked stimulating action on the production of opsonins, as well as on phagocytosis *in vitro*. They assert that there is also a certain specificity of action. Silver and gold are very active toward paratyphoid, copper toward the cholera vibrio, and platinum toward the streptococcus. This specificity is very interesting in view of the results which I have obtained on the germicidal action of oxidizing substances. I found that sodium iodoxybenzoate has different bactericidal powers against different organisms, being most active toward those organisms containing the least catalase. The catalase value seems to explain the specificity in the organisms which I studied. This work will soon be published. Since the colloidal metals are active oxidizing agents their effect on phagocytosis may be due to this property, just as the effect of sodium iodoxybenzoate is due to the oxygen in the molecule.

Collargol causes a leukocytosis with increase in the polymorphonuclears (Dunger). This leukocytosis is not accompanied by any increase in phagocytosis (Hoffman). According to Hamburger and Hekma colloidal silver (Crede) has no effect on phagocytosis *in vitro*. The colloidal solutions of various metals, as manganese, iron, copper, platinum, silver, gold, etc., exhibit properties closely resembling those of the catalases.

A. and L. Lumière and Chevrotier have confirmed the work of Trillat on the power of colloidal solutions of manganese to effect oxidations. According to these authors colloidal solutions of any metal capable of existing in two or more states of oxidation should exhibit properties similar to those of oxidases, the state of division of the colloidal substance determining the oxidizing power. Emulsions of iron or of manganese in albumen, gelatin, or gum have the power to blue guaiac, oxidize hydroquinon, pyrogallol, and paraphenylendiamin. They also found that the artificial oxidases protected animals against tetanus toxin. The work of Klebs, Linossier, Meyer, Bertrand, etc., makes it evident that certain metals, Fe, Mn, and Cu, enter into the composition of the oxygen-carriers of the living organism as essential ingredients. Robin and Bordet have arrived at the conclusion that the colloidal metals, especially manganese, promote oxidation in the tissues and intensify metabolism.

The bactericidal action of the colloidal metals is quite uncertain, as various investigators have obtained different results. M. Ascoli and G. Izar have given us an insight into the action of the metal hydrosols. Small amounts of the silver hydrosol activate the diastatic ferment of the liver and blood serum. Ascoli has further found that the metal hydrosols increase autolysis to varying degrees. This action can be inhibited by traces of HCN, $HgCl_2$, and CO, just as is their property to split H_2O_2. The colloidal metals in stable condition increase the nitrogen exchange, especially the nuclein metabolism (M. Ascoli and G. Izar). On intravenous injection silver hydrosol is found chiefly in the liver, bone marrow, and spleen. It is taken up by the Kupffer cells in the greatest amount.

ARSENICAL COMPOUNDS.

The investigations of Friedberger and Masuda indicate that the agglutinins are increased by salvarsan. The normal hemolysin is also increased. It is asserted that salvarsan noticeably increases the opsonin also two to eight hours after intravenous injection (Strubell). Boehncke found that salvarsan stimulates the production of antibodies. These results agree with those of Agazzi that various arsenic compounds stimulate antibody formation, and confirm the prediction of Ehrlich that arsenobenzol would stimulate the antibody-producing organs.

ALKALOIDS.

Only a few alkaloids have been studied for their effect on immune reactions. These are quinin, morphin, curare, and atropin. Manwaring and Ruh and Thomas Wilson found that quinin causes stimulation of phagocytosis in weak solution. According to Grünspan it has a slight stimulating effect in dilution of 0.002 per cent. Morphin has a depressing action (Reynolds). Cantacuzene demonstrated a detrimental action of opium on leukocytes and found that animals were rendered more susceptible to typhoid when given opium. Platania in 1889 found that frogs injected with curare were unable to overcome the subcutaneous injection of anthrax to which they are normally resistant.

ANESTHETICS AND SOPORIFICS.

Alcohol, chloroform, and ether have a detrimental action toward the natural defenses against infection (Rubin). They suspend phagocytosis *in vitro* (Snel). Ether reduces the property of phagocytosis in human blood (Graham). Laitinien found that alcohol hastens the death of experimentally infected animals. Similar results were obtained by Zimin, Abbot, and Krutschilin, for ether, chloroform, and alcohol. Snel found that chloral renders animals more susceptible to infection, but he did not study its effect *in vitro*.

The importance of the fats and lipoids in immune processes has been clearly demonstrated by Kyes, Neuberg, Morgenroth and Carpi, Woelfel, Vay, Noguchi, and others. Noguchi has even suggested that some of the complements of the serum may be salts of the higher fatty acids with weak organic acids. Hence substances which are solvents of those lipoids may be expected to have a marked effect on the immune reactions, as is the case with alcohol, ether, chloroform, etc. Graham found that ether given for anesthetic purposes reduces the property of phagocytosis, because of a direct action of the ether on the serum and the leukocytes. This action of the ether is probably due to its fat-solvent power. Lecithin counteracts this inhibitory effect of ether both *in vitro* and when given subcutaneously.

Likewise, as pointed out by Wells, the substances which precipitate fatty acids, such as calcium, barium, and strontium ions, inactivate the complement.

THE ACTION OF ANTIPYRETICS.

The action of antipyretics on phagocytosis has been investigated by Kentzler and V. Benczur. Quinin, antipyrin, phenacetin, pyramidon, and sodium salicylate have practically no effect. Quinin in typhoid, antipyrin in influenza, phenacetin in parametritis, pyramidon in pneumonia, and sodium salicylate in tonsillitis do not affect the phagocytic index.

LECITHIN AND CHOLESTEROL.

Kyes demonstrated that the hemolytic constituent of cobra venom could be complemented by lecithin. Morgenroth and Carpi found the same to be true for bee poison. Graham found that lecithin counteracts the inhibitory effect of ether on phagocytosis; it cannot act as opsonin in salt solution nor is it able to reactivate serum whose complement has been destroyed by heat. Walbum, who used a colloidal suspension, found cholesterol to have a marked stimulating effect on phagocytosis. I have repeated the experiments *in vitro* but with opposite results, as given below.

Finally Bechhold has studied the effect of some organic colloids on the serum (gelatin, peptone, dextrin, hemoglobin, egg albumin, pepsin, and pancreatin). Peptone is a very active stimulant of the leukocytes in a dilution of 1/160,000. Lactic acid in relatively large amount (n/40) causes destruction of the leukocytes. In dilutions above n/200 it seems to have no effect on phagocytosis in salt solution, serum, or whole blood. Bechhold found that lactic acid stimulated the phagocytosis of staphylococcus, and that both pepsin and pancreatin have a stimulating action in the presence of lactic acid. These results are not in accord with our observations on lactic acid, given below.

Neisser and Guerrini observed that a series of substances, toxic in large amount, are in small amount stimulants of the leukocytes. These so-called "leuko-stimulantien" are peptone (1/160,000), nucleic acid (1/100), quinin (1/12,800), potassium iodid (1/1,000), staphylolysin (1/500), alcohol (1/512). The leuko-stimulation occurs not only in immune serum but also in normal serum and even in the absence of opsonins. Peptone is the most active of these stimulants. Staphylolysin, a leukocyte poison, is in small amounts a stimulant of phagocytosis. Perhaps the bacteria, by the production of such substances, contribute to their own destruction. Furthermore, these results suggest that the stimulating action of some immune sera may be due to the presence not only of opsonins but also of leuko-stimulants, such as nucleic acid, peptone, etc.

TECHNIC OF STUDYING PHAGOCYTOSIS IN THE EXPERIMENTS NOW RECORDED.

The method employed in this work is essentially that of Wright and Douglas, with changes necessitated by the study of the action of the chemical substances on the process. Equal parts of human serum and the substance studied, and washed human leukocytes and the suspension of the bacterium studied are mixed and incubated at 37° C. for 15 minutes. Smears are then made in the same order as the mixtures in the pipettes were prepared, thus equalizing the time of action of the mixtures as much as possible. The number in any series is usually below eight or ten, thus making the time necessary for the preparation of the smears as short as possible. Controls, consisting of equal parts of human serum, and physiological salt solution in place of the drug studied, and washed human leukocytes, and the same suspension of the bacterium studied, are made at the beginning and at the end of each series. The average of these two counts is taken as the control count. The smears are made and stained with methyl-thionin. An average of 100 leukocytes is counted from each smear.

THE ACTION OF STRYCHNIN.

The fact that strychnin has a marked stimulating action on most animal organisms, and is widely used in various infectious diseases, led to the study of its action on phagocytosis. Strychnin has been shown to be less powerful in its action on leukocytes than any of the cinchona alkaloids. Strychnin has only one-fourth of the action of quinin toward infusoria. It accelerates the movements of medusae, and stimulates somewhat the movements of mollusca, but never causes any general tetanus in this animal. Its action in the vertebrates is well known.

Strychnin greatly increases the consumption of oxygen and the output of carbon dioxid, even when the muscular contraction has been previously eliminated by curare. The augmentation of oxidation in the tissues is accompanied by an increased formation of heat. Whether its stimulating action on phagocytosis, as shown by the following experiments, is due to its influence on oxidation, is difficult to determine, but the fact that alkaloids which depress oxidation also depress phagocytosis makes this a probable explanation.

The action of strychnin sulfate and nitrate on phagocytosis of streptococcus by human leukocytes was studied *in vitro*. The various dilutions of the salt were made in physiological salt solution. Further, leukocytes were treated with strychnin solution, washed free from the strychnin, and compared with untreated leukocytes. Finally, dilution experiments were carried out, using strychnin to dilute the serum in one series and salt solution in the other, and the dilution at which phagocytosis equals the spontaneous phagocytosis determined.

Serum	+salt solution	+leukocytes	+streptococcus	4.5
"	+1/10 per cent strychnin sulfate	+ "	+ "	1.5
"	+1/25 " " "	+ "	+ "	4.35
"	+1/75 " " "	+ "	+ "	6.24
"	+1/100 " " "	+ "	+ "	7.97

Serum	+salt solution	+leukocytes	+streptococcus	3.0
"	+1/250 per cent strychnin sulfate	+ "	+ "	4.5
"	+1/500 " " "	+ "	+ "	5.7
"	+1/1000 " " "	+ "	+ "	5.2
"	+1/2000 " " "	+ "	+ "	5.2

These results indicate that strychnin sulfate has a stimulating action on phagocytosis of streptococcus by human leukocytes in the presence of human serum. The action is probably on the opsonin of the serum, possibly by the formation of a more active opsonin-strychnin combination. That the action is not on the leukocytes is indicated by the following experiment:

Serum+leukocytic suspension treated with 1/500 per cent strychnin sulfate in salt solution for 20 minutes at 35° C., centrifuged, washed with salt solution +streptococcus:
Phagocytic index 1.5
Salt solution+leukocytic suspension (as above):
Phagocytic index 0.36
Serum+leukocytic suspension treated with salt solution only (as above):
Phagocytic index 1.4
Salt solution+leukocytic suspension treated with salt solution only (as above):
Phagocytic index 0.34

Here we see that the phagocytic index with leukocytes treated with strychnin sulfate was the same as that with leukocytes not treated with strychnin solution, indicating that there is no action on the leukocytes in the solution used. That the action is on the serum is indicated by the results of dilution experiments:

Salt Solution Dilutions.		Strychnin Sulfate Dilutions (1/500 per cent).	
Serum	1.0	Serum	1.0
Serum /4	0.52	Serum /4	0.82
Serum /8	0.32	Serum /8	0.56
Serum /32	0.28	Serum /32	0.36
Serum /64	0.0	Serum /128	0.18
Salt solution	0.26	Salt solution	0.20

Hence with strychnin sulfate the dilution required to stop phagocytosis is greater than with physiological salt solution, indicating that the action is on the opsonin of the serum.

A series of experiments with strychnin nitrate shows a similar stimulating action on phagocytosis. Here also the action is on the serum and not on the leukocytes. In the absence of serum the strychnin has practically no action. Since the mixtures in these experiments consist of four equal parts, the dilution of the strychnin solution is four times that of the solution used. Hence the strychnin sulfate has its most marked stimulating effect at 1/2000 per cent and the action of 1/8000 per cent is almost as great. This action of strychnin offers a possible explanation of its value in various infectious diseases and suggests a more extended use of its preparations in such diseases.

THE ACTION OF MORPHIN.

The following experiments were made with morphin to test its effect on phagocytosis:

Serum +salt solution	+	leukocytes	+	streptococci	4.70
Salt solution+salt solution	+	"	+	"	0.06
Serum +1 per cent morphin sulfate	+	"	+	"	2.02
" +1/10 " " "	+	"	+	"	2.36
" +1/50 " " "	+	"	+	"	3.98
" +1/100 " " "	+	"	+	"	4.02
" +1/1000 " " "	+	"	+	"	4.40

Serum+leukocytic suspension treated with 1/10 per cent morphin sulfate in salt solution for 30 minutes at 35° C., centrifuged, washed with salt solution+streptococci:

Phagocytic index........ 8.1

Serum+leukocytic suspension treated with salt solution only as above+streptococci:

Phagocytic index........ 12.7

These results indicate that morphin sulfate has a depressing action on phagocytosis of streptococci by human leukocytes up to 1/100 per cent and to a slight degree up to 1/1000 per cent. This action is largely on the leukocytes, for leukocytes treated with 1/10 per cent morphin sulfate gave an index of 0.63 as compared with 1.0 for leukocytes treated only with salt solution. That this depressing action is of considerable importance in infection has already been demonstrated by animal experimentation. Animals given opium in various forms, as well as morphin itself, succumb more readily to anthrax and other infectious diseases than normal animals (Cantacuzene, Oppel, and Gheorghiewski).

THE ACTION OF CAFFEIN.

In the study of the action of caffein the pure alkaloid was used, as it, unlike most of the alkaloids, is soluble in water. All of the dilutions were made in normal salt solution:

Serum +salt solution	+leukocytes+streptococci	2.8
Salt solution+salt solution	+ " + "	0.06
Serum +caffein 1 per cent	+ " + "	0.36
" + " 1/10 "	+ " + "	2.46
" + " 1/100 "	+ " + "	2.74
" + " 1/500 "	+ " + "	2.94
" + " 1/1000 "	+ " + "	2.70
" + " 1/2000 "	+ " + "	2.70

Serum+salt solution	+leukocytes+streptococci	3.64
Salt solution+salt solution	+ " + "	0.2
" " +caffein 1/100 per cent	+leukocytes + streptococci	0.2
" " + " 1/500 "	+ + "	0.26
" " + " 1/1000 "	+ + "	0.1

The foregoing results indicate that caffein has a depressant effect on phagocytosis up to 1/10 per cent. In dilutions greater than this it has practically no action, there being no effect on the leukocytes or on the opsonin of the serum. The widespread use of preparations of caffein as beverages makes it a substance of considerable interest. Caffein, unlike alcohol, ether, opium, strychnin, etc., induces no marked symptoms in moderate quantities, and unlike these it has no marked effect on phagocytosis.

THE ACTION OF CHLORAL.

Chloral has the same general action as the anesthetics, hence it is interesting to learn its effect on phagocytosis and compare it with the action of the anesthetics. The effects of chloral on the tissues have been found to correspond closely to those of chloroform. Imperfect oxidation is produced, accompanied by an increase in unoxidized sulfur in the urine; less oxygen is absorbed and less carbon dioxid excreted (Cushny).

Like chloroform, chloral has antiseptic properties, produces fatty changes, and causes depression; unlike chloroform, it has a local irritant action, and causes no stage of stimulation.

The results with chloral follow:

Serum +salt solution	+leukocytes+streptococci	1.7
Salt solution+salt solution	+ " + "	0.2
Serum +chloral 1/50 per cent	+ " + "	1.0
" + " 1/100 "	+ " + "	1.3
" + " 1/500 "	+ " + "	1.4

Hence chloral hydrate in a dilution of 1 to 5,000 has a distinct inhibitory effect on phagocytosis and this is present even in 1 to 50,000. It may, therefore, be of importance in infections not to prescribe large amounts of chloral, for by so doing the phagocytic power may be reduced, as has already been demonstrated by Snel for infected animals.

THE ACTION OF CHOLESTEROL.

I have repeated the experiments of Walbum, using the colloidal suspension of cholesterol made according to Porges and Neubauer, i.e., first dissolving the cholesterol in acetone and gradually adding the acetone solution, with constant stirring, to the required amount of distilled water. The acetone is removed by evaporation at a low temperature, and the suspension then filtered. The suspension used was N/2000, as employed by Walbum (0.193 gm. per liter).

Serum+leukocytes+streptococci	2.74
Cholesterol+leukocytes+streptococci	1.54
Salt solution+leukocytes+streptococci	0.52

Serum+cholesterol+leukocytes+streptococci	1.18
Salt solution+cholesterol+leukocytes+streptococci	1.94

These results indicate that cholesterol has an inhibitory action on phagocytosis in the presence of serum *in vitro*. This can be explained by action on the serum, the cholesterol probably combining with the opsonin as it is known to combine with various lysins. This combination is less active than the cholesterol itself. For we see in the above experiments that cholesterol is capable of replacing the opsonin of the serum to a certain extent, cholesterol giving a higher phagocytic index than salt solution. It does not completely replace the opsonin in the dilutions used in these experiments; the index of the serum is higher than with the colloidal cholesterol alone.

That this action of cholesterol may be due to its colloidal condition cannot be denied, altho, according to Walbum, colloidal suspensions of the aliphatic alcohols, cetyl, and myricycl alcohol are inactive. Walbum found that cholesterol gave a higher phagocytic index with the colon bacillus than normal human serum. This is not in accord with our results with the streptococcus.

THE ACTION OF ANTIPYRIN.

Serum+salt solution	+leukocytes	+streptococci	3.1
Salt solution+salt solution	+ "	+ "	0.4
Serum +antipyrin 1 per cent	+ "	+ "	2.6
" + " 1/10 "	+ "	+ "	3.4
" + " 1/50 "	+ "	+ "	3.7
" + " 1/100 "	+ "	+ "	3.2

These results indicate that antipyrin has a slight stimulating action on phagocytosis in dilutions of 1/10 to 1/50 per cent. Above this dilution it has no effect. It has been observed by Schultze and Beniasch that the use of the antipyretics does not retard the formation of the protective substances, for in infected animals treated with enormous quantities of antipyrin the serum displayed the same agglutinating properties as that of the control animals not subjected to the drug. In this connection may be mentioned the results of Kentzler and v. Benczur, who found that the antipyretics in solutions of 1/100, 1/500, 1/1,000 have practically no effect on the phagocytosis of staphylococci *in vitro*. In animals and in man they cause a slight and transient rise of phagocytosis or no effect at all.

THE ACTION OF LACTIC ACID.

In certain experiments, the records of which were placed at my disposal by Dr. Hektoen, lactic acid appears to suspend hemolysis and phagocytosis by acting on the complement and the opsonin. Thus 0.001 c.c. of a concentrated lactic acid (77.14 per cent $C_3H_6O_3$ by weight) prevents all hemolytic effect of 0.2 c.c. of dog serum upon guinea-pig corpuscles (total quantity in each tube being

2 c.c.); and 0.0006 of this acid neutralizes the double lytic dose of normal guinea-pig serum (complement) for 1 c.c. of a 5 per cent suspension of washed rabbit corpuscles sensitized in heated, immune, guinea-pig serum. The dilution of lactic acid in the above experiments is 1 in 2,596 and 1 in 4,320.

Lactic acid in doses of 0.003–0.0015 prevents phagocytosis of anthrax bacilli and staphylococci by the leukocytes in 0.5 c.c. of human blood. The result of the following experiments indicates that this action is due to neutralization or destruction of the opsonin. 0.2 c.c. of serum is mixed with 0.0015 c.c. of lactic acid in 0.5 c.c. of salt solution and placed at 36° C. for one hour; anthrax bacilli are then added and the tube returned to the incubator for 30 minutes; at this time the bacilli are washed twice and suspended in 0.5 c.c. of salt solution to which is added 0.5 c.c. of a suspension of homologous washed corpuscles. As control, mixtures prepared in the same way, except that the bacilli are treated with serum free from lactic acid, are used. Counting 50 leukocytes in each case with special reference to phagocytosis gives the following result:

	Phagocytosis	
	+	0
Bacilli sensitized in dog serum+lactic acid	0	50
" " " " " +NaCl only	45	5
" " " human serum+lactic acid	0	50
" " " " " +NaCl only	38	12

Clearly the lactic acid in some way prevents the serum from making the bacilli susceptible to phagocytosis. In full accord with the anti-phagocytic effect of lactic acid is its neutralization of the anthracidal action of defibrinated dog blood as shown in the following table:

Defibrinated Dog Blood 0.5 c.c. +Lactic Acid +NaCl Solution to Make 1 c.c.	No. of Bacilli in Total Quantity		
	At Once	3 Hrs.	6 Hrs.
Lactic acid 0.003	5,880	0	0
" " 0.0015	"	800	4,270
" " 0.00075	"	6,370	3,080
" " 0.000375	"	600	88
NaCl only+blood	"	548	22

SUMMARY.

From the results of experiments recorded in the literature and the results I have obtained it appears that the effect of chemical substances on phagocytosis varies with their chemical constitution and their pharmacological action.

Alcohol, chloroform, ether, chloral, morphin, and potassium cyanid depress phagocytosis *in vitro*. The substances all have an inhibitory effect on oxidation, to which their action on phagocytosis is in all probability due.

That oxidative processes play a rôle in phagocytosis is definitely shown by the fact that sodium iodoxybenzoate, which is an organic peroxid and owes its pharmacological action as well as germicidal action to the presence of physiologically active oxygen attached to the iodin atom in its molecule, has a marked stimulating effect on the phagocytosis of streptococci and staphylococci *in vitro*, as I have previously demonstrated. The action appears to be on the opsonin of the serum, which is rendered more active. Likewise strychnin and certain colloidal metals which have a stimulating effect on oxidative processes stimulate phagocytosis to a marked degree, as shown in this report. The beneficial effects of improved oxygenation of the blood in infectious diseases may be due in part to the stimulating action on phagocytosis. Furthermore, substances which have a depressing effect on oxidations, as potassium cyanid, chloroform, ether, morphin, germicides, etc., have a distinct detrimental effect on phagocytosis as well as other immune reactions.

Caffein and the antipyretics which are more inert drugs have little, if any, action on phagocytosis. They have no known effect on oxidations in the body.

The salts are mostly injurious to phagocytosis, to an extent depending on their toxicity, their ability to effect an exchange of ions with the salts in the cells, and their ability to combine with the opsonins.

Those substances which depress phagocytosis *in vitro* have been found, when studied *in vivo*, to have a detrimental effect on the immunity reactions, e.g., the anesthetics, chloral, morphin, some salts, and the antiseptics. The substances which have been found to stimulate phagocytosis at all are calcium, magnesium, and mercuric chlorids; quinin; colloidal metals; peptone in dilute solution; nucleic acid; a few other leuko-stimulants such as staphylolysin in weak solution, potassium iodid, and perhaps alcohol; strychnin sulfate and nitrate; sodium iodoxybenzoate; salvarsan and other arsenic compounds; and possibly certain colloidal suspensions of cholesterol. It is interesting that all of these which have been studied *in vivo* have also had a favorable action, namely, salvarsan, calcium chlorid, quinin, colloidal metals (platinum, silver, gold, copper), mercuric chlorid in weak solutions, sodium iodoxybenzoate (results not yet published), and cholesterol in colloidal suspension (Walbum). Hence the action of a chemical substance on phagocytosis *in vitro* is a good index of the effect of that substance when used *in vivo*.

The mechanism of the action of chemical substances on opsonin has never been carefully worked out. It has been shown conclusively that the opsonic power of a serum is dependent upon a thermostable element, the activity of which is greatly increased by a coexistent thermolabile complement-like substance. This is true of normal as well as of immune serum; in the latter the thermostable element is increased. Whether the chemical substances act on the specific thermostable element which unites firmly with the object of its action, or on the thermolabile element, remains to be determined.

Strychnin has a marked stimulating action on the phagocytosis of streptococci by human leukocytes in the presence of human serum. The stimulating action is probably on the opsonin of the serum by the formation of a more active opsonin-strychnin combination. Whether the action is on both elements of the opsonin or on either one remains to be determined. The action of strychnin on phagocytosis offers an explanation of its value in infections, and suggests a more extended use of this drug. The interaction of the specific antibody and the chemical substance is in this case a beneficial one, and emphasizes the possibility of a combined therapy against the infectious diseases, the antibodies produced in the body being increased in amount by drugs introduced from without or their action on the invading microorganism accelerated.

Morphin has a depressing effect on the phagocytosis of streptococci by human leukocytes in the presence of human serum. The effect is largely on the leukocytes. As is generally assumed, large doses of morphin lower the resistance to infection, hence it should be used cautiously in bacterial diseases.

Caffein has practically no effect on the phagocytosis of streptococci by human leukocytes in the presence of human serum, in dilutions greater than 1/10 per cent. Stronger solutions have an inhibitory effect. It is interesting that this alkaloid, unlike morphin or strychnin, produces no marked symptoms when given internally in moderate quantities, nor has it any effect on oxidative processes.

Chloral has a distinct inhibitory effect on the phagocytosis of streptococci by human leukocytes in the presence of human serum. That this detrimental action is of importance in infection has already been demonstrated.

Cholesterol in colloidal suspension is capable of replacing the opsonin of the serum, but not completely, in the suspension used (n/2,000). It causes some phagocytosis of streptococci by human leukocytes in the absence of serum. The phagocytosis is not as great as that produced by the normal serum. Cholesterol reduces the opsonic power of normal serum, probably by combining with the opsonin. This combination of cholesterol with serum is less active than either the serum alone or the cholesterol alone.

Antipyrin has no effect on the phagocytosis of streptococci by human leukocytes in the presence of human serum. The antipyretics have been shown to have no effect on the formation of protective substances *in vivo.*

Lactic acid suspends hemolysis and phagocytosis by acting on the complement and the opsonin. This action occurs in dilutions of 1–2,000 to 1–4,000. The lactic acid prevents the serum from making the bacilli (anthrax) susceptible to phagocytosis. Lactic acid has not only an antiphagocytic effect but it also neutralizes the anthracidal action of defibrinated dog blood.

The effects of an agent on the processes of immunity should be considered carefully when the use of that agent seems indicated in infectious diseases. The possibility of a combined therapy, consisting of the use of immune sera or vaccines together with certain drugs, suggests itself in the case of drugs which have themselves a stimulating effect on phagocytosis. The drugs may act as leukostimulants, or increase the production of antibodies, or combine with the antibodies to render them more active.

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